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COMPARATIVE STUDY OF CYTOCHROME P-450 IN LIVER MICROSOMES

A FORM OF MONKEY CYTOCHROME P-450, P-450-MK1, IMMUNOCHEMICALLY CROSS-REACTIVE WITH ANTIBODIES TO RAT P-450-MALE

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Abstract—Cytochrome P-450, designated as P-450-MK1, which is cross-reactive with antibodies to rat P-450-male, was purified to an electrophoretical homogeneity from liver microsomes of the untreated male crab-eating monkey.

The molecular weight of P-450-MK1 was estimated to be 50,000 by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). The oxidized form of P-450-MK1 showed a peak at 418 nm, indicating that this cytochrome is in a low spin state. The carbon monoxide-bound reduced form showed a peak at 451 nm. The first 22 amino acid residues of the NH_2-terminal sequence of P-450-MK1 was fairly homologous to those of P-450-male (75% identity, not including unidentified amino acid residues).

Unlike the P-450-male, P-450-MK1 did not exhibit catalytic activities for testosterone 2α- and 16α-hydroxylations and catalyzed testosterone 6β-hydroxylation. It is, therefore, suggested that although the spectral and immunochemical properties and the *N*-terminal amino acid sequence of P-450-MK1 were similar to those of P-450-male, the physiological functions of P-450-MK1 may be somewhat different from those of P-450-male.

Comparison of the physico-chemical properties of P-450-MK1 with those of P-450-D1 and P-450-HM2, which are cross-reactive with anti-P-450-male antibodies, purified from liver microsomes of dogs and humans, respectively, are also discussed.

The hepatic microsomal cytochrome P-450 plays an important role as a terminal oxidase of the monooxygenase system which functions in the metabolism of a wide variety of endogenous and exogenous substrates, and constitutes a superfamily of enzymes, comprising some 20 to 30 families [1]. Multiple forms of cytochrome P-450 have been purified from liver microsomes of untreated or drug-treated experimental animals. With respect to cytochrome P-450 inducible by chemicals and drugs, transinterspecies homology has been recently shown to be present among forms of cytochrome P-450 in catalytic activity, immunochemical reactivity and amino acid sequence deduced from base sequence analysis of cDNA clones. For example, it has been shown that P-450c purified from rats treated with 3-methylcholanthrene [2] or β-naphthoflavone [3] is homologous to rabbit P-450 LM_6 [4, 5] and mouse P_1-450 [6]. In addition, P-450d purified from rats treated with 3-methylcholanthrene [7] has been demonstrated to be similar to rabbit LM_4 [8] and mouse P_3-450 [6].† Furthermore, the amino terminal sequence of P-450 TAO or P-450p purified from rats treated with macrolide antibiotics or PCN has been shown to be highly homologous to that of rabbit P-450 LM_{3c} [9].‡ It is, however, unknown whether transinterspecies homology among constitutive forms of cytochrome P-450 is present. Kamataki *et al.* [10] purified a male specific form of cytochrome P-450, named P-450-male,§ from liver microsomes of untreated male rats. Thus, it was of special interest to investigate whether or not a cytochrome P-450 homologous to P-450-male in liver microsomes of untreated male rats is present in liver microsomes of untreated male crab-eating monkeys. In this paper, we purified a form of cytochrome P-450, P-450-MK1, cross-reactive with anti-P-450-male antibodies, from liver microsomes of the male crab-eating monkey.

MATERIALS AND METHODS

Materials. NADP, NADPH, glucose-6-phosphate and glucose-6-phosphate dehydrogenase were purchased from Oriental Yeast, Tokyo, Japan. Mephenytoin and Emulgen 911 were kindly provided by Professor Hirobe, Faculty of Pharmaceutical Sciences, Tokyo University, and Kao Atlas Co. (Tokyo, Japan), respectively. Testosterone and progesterone were from Wako Pure Chemical Industries, (Osaka, Japan), and the hydroxylated metabolites of testosterone and progesterone were from Steraloids Inc. (Wilton, NH) and Sigma (St. Louis, MO). Preparative DEAE-5PW column and

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† P-450c, LM_6, P_1-450, P-450d, LM, and P_3-450 belong to the P-450 I family.

‡ P-450 TAO, P-450p, and LM_{3c} belong to the P-450 III family.

§ P-450-male belongs to the P-450 II family.

analytical CM-5PW column were purchased from Toyo Soda (Tokyo, Japan), and analytical ES-502C column from Asahi Kasei Industry (Japan). Hydroxylapatite and Sepharose 4B were supplied from BioRad and Pharmacia (Uppsala, Sweden), respectively. Other chemicals used were of the highest grade commercially available.

Purification of cytochrome P-450 (P-450-MK1) from liver microsomes of male crab-eating monkeys. Male crab-eating monkeys weighing about 2.5 kg were used for purification. Cytochrome P-450 was solubilized with sodium cholate [11] and was applied onto a ω-aminooctyl Sepharose 4B column (2.6 × 60 cm). The column was washed with 10 mM potassium phosphate (pH 7.4) containing 20% glycerol, 1 mM DTT, 0.1 mM EDTA and 0.5% sodium cholate (buffer A), subsequently washed with buffer A containing 0.2% Emulgen 911 (buffer B) at a flow rate of 30 ml/hr. After the column was washed with three times the column volume of buffer B, a portion of ω-aminooctyl Sepharose 4B resin which apparently contained cytochrome P-450 was cut off and washed with buffer B. Cytochrome P-450 extracted with buffer B was found to exhibit cross-reactivity with anti-P-450-male antibodies by Western blot analysis. Purification of P-450-male from liver microsomes of untreated rats and preparation of antibodies to purified P-450-male were performed as described previously [10]. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot-peroxidase anti-peroxidase staining were carried out according to the method of Laemmli [12] and Guengerich *et al.* [13], respectively. The fraction containing cytochrome P-450 immunochemically cross-reactive with anti-P-450-male antibodies was concentrated by ultrafiltration on a UK-50 membrane (Toyo Roshi, Tokyo, Japan) and then diluted with 20 mM Tris–acetate (pH 7.4) containing 20% glycerol. These concentrations and dilutions were repeated at least four times until the concentration of sodium cholate was less than 0.01%. Further purification of cytochrome P-450 was conducted using high-performance liquid chromatography (HPLC) equipped with a preparative DEAE-5PW (21.5 × 150 mm) as described previously [14]. Cytochrome P-450, which is immunochemically cross-reactive with anti-P-450-male antibodies, was eluted from the column near the void volume by washing with 20 mM Tris–acetate (pH 7.5) containing 20% glycerol and 0.4% Emulgen 911 at a flow rate of 1 ml/min. Fractions containing cytochrome P-450 were pooled and then applied onto an analytical ES-502C column (7.5 × 74 mm) which had been equilibrated with 20 mM sodium phosphate (pH 6.5) containing 20% glycerol and 0.4% Emulgen 911 (buffer C). Cytochrome P-450 was eluted from the column by washing with a linear gradient of sodium acetate concentration (100–300 mM at a flow rate of 0.5 ml/min) in a total volume of 60 ml. Fractions containing cytochrome P-450, which is immunochemically cross-reactive with anti-P-450-male antibodies, were pooled, and then applied onto a hydroxylapatite column (5 × 5 mm) to remove Emulgen 911. After the hydroxylapatite column was thoroughly washed with 10 mM potassium phosphate (pH 7.4) containing 20% glycerol, cytochrome P-450 was eluted from the column with 300 mM potassium phosphate (pH 7.4) containing 20% glycerol.

Reconstitution of monooxygenase system. A typical incubation mixture contained 100 mM sodium phosphate (pH 7.4), 50 μM EDTA, cytochrome P-450 (0.1 nmol for the assay of testosterone and progesterone hydroxylase activities, 0.05 nmol for the assay of aniline hydroxylase, aminopyrine *N*-demethylase, 7-ethoxycoumarin *O*-deethylase and mephenytoin hydroxylase and *N*-demethylase activities), 0.5 unit of NADPH-cytochrome P-450 reductase, 20 μg of dilauroyl-L-3-phosphatidyl choline, NADPH-generating system (0.5 mM NADP, 5 mM glucose-6-phosphate, 1 unit of glucose-6-phosphate dehydrogenase and 5 mM $MgCl_2$) and a substrate in a final volume of 0.5 ml. In the case of reconstituted system for the assay of testosterone and progesterone hydroxylase activities, 0.2 mg of sodium cholate was added to the system described above.

Assays. The activities of aniline hydroxylase, aminopyrine *N*-demethylase and 7-ethoxycoumarin *O*-deethylase were measured by the methods of Imai *et al.* [15], Nash [16] and Aitio [17], respectively. Mephenytoin oxidase activity was determined according to the method of Meier *et al.* [18] with minor modifications. Activities of testosterone and progesterone hydroxylases were measured by the methods of Hayashi *et al.* [19] and Miura *et al.* [20], respectively.

Other methods. P-450-HM2* and P-450-D1,† which are immunochemically cross-reactive with anti-P-450-male antibodies, were purified from liver microsomes of human and untreated beagle dogs, respectively. NADPH-cytochrome P-450 reductase was purified from liver microsomes of phenobarbital-treated rats according to the method of Yasukochi and Masters [21]. Cytochrome P-450 was measured according to the method of Omura and Sato [22]. The activity of NADPH-cytochrome P-450 reductase was determined by the method of Phillips and Langdon [23] using cytochrome *c* as an electron acceptor. One unit of the reductase was defined as the amounts of the enzyme catalyzing the reduction of one μmole of cytochrome *c* per min. Protein concentration was estimated according to the method of Lowry *et al.* [24] using bovine serum albumin as the standard. The N-terminal amino acid sequence of P-450-MK1 was analyzed by Edman degradation using automatic gas phase sequencer (Applied Bio-systems, Model 477A) after P-450-MK1 was dialyzed thoroughly against water and lyophilized.

RESULTS

Table 1 shows the summary of the purification of P-450-MK1 from liver microsomes of untreated male monkeys. Specific content of the final preparation of P-450-MK1 was 12.5 nmole/mg of protein, and the recovery of the cytochrome was approximately

* Purification of P-450-HM2 will be reported elsewhere (submitted for publication).

† Purification and characterization of P-450-D1 was described in the revised manuscript for publication in *Biochem. Pharmacol.*

Table 1. Purification of P-450-MK1 from liver microsomes of untreated male crab-eating monkeys

	Protein (mg)	Cytochrome P-450 T.C.* (nmoles)	S.C.† (nmole/mg)	Recovery (%)
Microsomes	1920	1480	0.77	100
Solubilized supernatant	1840	1200	0.65	81
Aminooctyl Sepharose 4B eluate	167	317	1.90	14.5
DEAE-5PW eluate	5.5	30.6	5.6	2.1
ES-502C eluate	1.3	9.8	7.5	0.7
Hydroxylapatite eluate (P-450-MK1)	0.16	2.0	12.5	0.13

* Total content.
† Specific content.

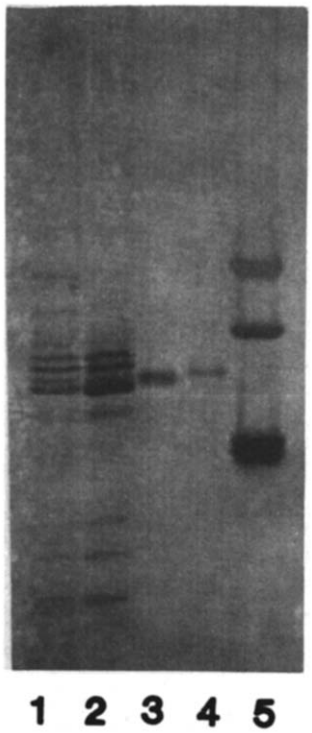

Fig. 1. SDS-PAGE of P-450-MK1 purified from liver microsomes of untreated male crab-eating monkeys. Lanes 1–5 contained monkey liver microsomes, aminooctyl Sepharose 4B column eluate, purified P-450-MK1, purified P-450-male and standard proteins (bovine serum albumin, catalase and aldorase), respectively.

0.13% of total cytochrome P-450 in liver microsomes. As shown in Fig. 1, the final preparation of P-450-MK1 gave a single protein band on SDS-PAGE. When compared to standard proteins (bovine serum albumin, catalase and aldorase), an apparent minimum molecular weight of P-450-MK1 was estimated to be 50,000. The carbon monoxide difference spectrum of P-450-MK1 showed a peak at 451 nm. The oxidized form of the cytochrome showed peaks at 567, 538 and 418 nm, suggesting that P-450-MK1 is in a low spin state. The drug oxidation activities of P-450-MK1 in a reconstituted system were compared to those of P-450-HM2, P-450-D1 and P-450-male which were purified from liver microsomes of adult humans, beagle dogs and rats, respectively, according to the cross-reactivity with anti-P-450-male antibodies (Table 2). Catalytic activities of P-450-MK1 for aminopyrine and mephenytoin *N*-demethylations were comparable to those of P-450-HM2, P-450-D1 and P-450-male. Furthermore, *N*-demethylaion but not *p*-hydroxylation was a major common oxidative metabolic pathway of mephenytoin with cytochrome P-450 purified from all animal species examined. Aniline hydroxylase activity of P-450-MK1 was about 8-fold less than that of P-450-D1. On the contrary, P-450-MK1 was about 6-times more active in 7-ethoxycoumarin *O*-deethylation than P-450-HM2. The catalytic activities of P-450-MK1 for testosterone and progesterone hydroxylations were also compared to those of cytochrome P-450 purified from other animal species (Table 3). The formation of androstenedione was found in all purified preparations of cytochrome P-450 to varying extents. It was of interest to note that the position of hydroxylation was dependent on the form of cytochrome P-450 used. As expected, P-450-male preferentially catalyzed testosterone hydroxylation at positions of 2α and 16α. However, these hydroxylase activities were not detectable in a reconstituted system containing P-450-HM2, P-450-MK1 or P-450-D1, except that P-450-D1 catalyzed the 16α-hydroxylation of testosterone at a comparable rate to P-450-male. P-450-MK1 catalyzed the 2α-hydroxylation of testosterone at a slow but a detectable rate. P-450-MK1 did not show detectable activities for progesterone hydroxylations studied.

It is well known that N-terminal amino acid sequences vary among forms of cytochrome P-450. Thus, the sequences of these forms of cytochrome P-450 were compared with each other. As can be seen in Fig. 2, the first 22 amino terminal sequence of P-450-MK1 was found to be highly homologous to that of P-450-male and P-450-HM2 but to a lesser extent than those of P-450-D1.

DISCUSSION

In the present study, P-450-MK1 immuno-

Table 2. Comparison of drug metabolizing enzyme activities of P-450-MK1 with those of cytochrome P-450 immunochemically related to rat P-450-male in other species

	Cytochrome P-450			
	P-450-MK1	P-450-HM2	P-450-D1	P-450-male
	(nmole/min/nmole P-450)			
Aniline *p*-hydroxylation	0.98	*	7.54	1.56
Aminopyrine *N*-demethylation	12.2	9.90	11.5	11.5
7-Ethoxycoumarin *O*-deethylation	1.07	0.19	1.27	0.68
Mephenytoin *p*-hydroxylation	N.D.	N.D.	*	0.08
Mephenytoin *N*-demethylation	1.70	1.64	*	1.58

* Not measured. N.D. not detectable. P-450-MK1, P-450-HM2, P-450-D1 and P-450-male were purified from liver microsomes of monkeys, humans, dogs and rats, respectively, and were cross-reactive with anti-P-450-male antibodies.

Table 3. Testosterone and progesterone hydroxylation activities of cytochrome P-450 from different species

	Cytochrome P-450			
	P-450-MK1	P-450-HM2	P-450-D1	P-450-male
	(nmole/min/nmole P-450)			
Testosterone				
2α-hydroxylation	N.D.	N.D.	N.D.	3.58
6α-hydroxylation	0.01	N.D.	N.D.	N.D.
6β-hydroxylation	N.D.	N.D.	N.D.	0.82
16α-hydroxylation	N.D.	N.D.	4.20	3.91
16β-hydroxylation	N.D.	0.01	N.D.	N.D.
Androstenedione formation	0.24	0.47	4.51	9.26
Progesterone				
2α-hydroxylation	N.D.	N.D.	N.D.	2.49
6β-hydroxylation	N.D.	N.D.	0.36	0.42
16α-hydroxylation	N.D.	N.D.	2.76	2.70
21-hydroxylation	N.D.	N.D.	2.98	N.D.

N.D. not detectable. P-450-MK1, P-450-HM2, P-450-D1 and P-450-male were purified from liver microsomes of monkeys, humans, dogs and rats, respectively, and were cross-reactive with anti-P-450-male antibodies.

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                1               5                  10                  15                  20
P-450-MK1  :  Met-Asp-Pro-Phe-Val-Val-Leu-Val-Leu- X -Leu-Ser-Phe-Val-Leu-Leu-Phe-Ser-Leu- X -Arg-Gln-
P-450-HM2  :  Met-Asp-Ser-Leu-Val-Val-Leu-Val-Leu- X -Leu-Ser- X -Leu-Leu-Leu-Leu-Ser-Leu-Trp-Arg-Gln-
P-450-D1   :  X - X -Leu-Phe-Ile-Val-Leu-Val-Ile- X -Leu-Ser- X -Leu-Ile-Ser-Phe-Phe-Leu-Trp-Asn-Gln-
P-450-male :  Met-Asp-Pro-Val-Leu-Val-Leu-Val-Leu-Thr-Leu-Ser-Ser-Leu-Leu-Leu-Leu-Ser-Leu-Trp-Arg-Gln-
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Fig. 2. The N-terminal amino acid sequences of cytochrome P-450 cross-reactive with anti-P-450-male antibodies in different species. X represents unidentified amino acid residues. P-450-MK1, P-450-HM2, P-450-D1 and P-450-male were purified from liver microsomes of monkeys, humans, dogs and rats, respectively, and were cross-reactive with anti-P-450-male antibodies.

chemically cross-reactive with anti-P-450-male antibodies has been purified from liver microsomes of untreated male crab-eating monkeys to an electrophoretical homogeneity using HPLC techniques as described by Funae and Imaoka [14]. The molecular weight of P-450-MK1 (50 kDa) was somewhat different from that of P-450-D1 (47 kDa), P-450-male (51 kDa) or P-450-HM2 (54 kDa), which are also cross-reactive with anti-P-450-male antibodies. However, spectral properties and the N-terminal amino acid sequence of P-450-MK1 were similar to those of P-450-HM2, P-450-D1 and P-450-male.

It is interesting to note that the catalytic properties of P-450-MK1 for monooxygenase reactions were somewhat qualitatively similar to those of P-450-HM2 purified from human liver microsomes rather than those of P-450-D1 and P-450-male purified from liver microsomes of dogs and rats, respectively.

Ohmori *et al.* [25] previously purified a form of cytochrome P-450 (designated as monkey P-450) from liver microsomes of untreated monkeys. Some similarities were observed between P-450-MK1 and monkey P-450 in their molecular weights, and catalytic activities for aniline hydroxylation and 7-ethoxycoumarin *O*-deethylation. However, since neither the N-terminal amino acid sequence nor the catalytic activities of monkey P-450 for testosterone and progesterone hydroxylations have been reported, the identity between the monkey P-450 and P-450-MK1 cannot be confirmed at present.

P-450-D1 showed a high similarity to P-450-male in its structural and catalytic properties.

During the course of these experiments, we found that at least two other forms of cytochrome P-450, which were cross-reactive with anti-P-450-male antibodies, were present in liver microsomes of monkeys. The content of these forms of cytochrome P-450 was not sufficient to purify in the present study. It is unclear whether the cytochrome(s) P-450 which is similar to P-450-male in both structural and catalytic properties is present in monkey liver microsomes.

REFERENCES

1. Nebert DW and Gonzalez FJ, P450 gene: Structure, evolution, and regulation. *Annu Rev Biochem* **56**: 945–993, 1987.
2. Ryan DE, Thomas PE, Korzeniowski D and Levin W, Separation and characterization of highly purified forms of liver microsomal cytochrome P-450 from rats treated with polychlorinated biphenyls, phenobarbital and 3-methylcholanthrene. *J Biol Chem* **254**: 1365–1374, 1979.
3. Norman RL, Johnson EF and Muller-Eberhard V, Identification of the major cytochrome P-450 form transplacentally induced in neonatal rabbits by 2,3,7,8-tetrachlorodibenzo-p-dioxin. *J Biol Chem* **253**: 8640–8647, 1978.
4. Okino ST, Quattrochi LC, Barnes HJ, Osanto S, Griffin KJ, Johnson EF and Tukey RH, Cloning and characterization of cDNA encoding 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-inducible rabbit mRNAs for cytochrome P-450 isozyme 4 and 6. *Proc Natl Acad Sci USA* **82**: 5310–5314, 1985.
5. Coon MJ, Black SP, Koop DR, Morgan ET and Tarr GE, Structural and catalytical studies with purified microsomal enzymes. In: *Microsomes, Drug Oxidations, and Drug Toxicity* (Eds. Sato R and Kato R), pp. 13–23. Scientific Societies Press, Tokyo, 1982.
6. Gonzalez FJ, MacKenzie PI, Kimura S and Nebert DW, Isolation and characterization of full-length mouse cDNA and genomic clones of 3-methylcholanthrene-inducible cytochrome P_1-450 and P_3-450. *Gene* **291**: 281–292, 1984.
7. Sogawa K, Gotoh O, Kawajiri K, Harada T and Fujii-Kuriyama Y, Complete nucleotide sequence of a methylcholanthrene inducible cytochrome P-450 (P-450d) gene in the rat. *J Biol Chem* **260**: 5026–5032, 1985.
8. Ozols J, Complete amino acid sequence of a cytochrome P-450 isolated from β-naphthoflavone-induced rabbit liver microsomes. Comparison with phenobarbital-induced and constitutive isozymes and identification of invariant residues. *J Biol Chem* **261**: 3965–3979, 1986.
9. Wrighton SA, Maurel P, Schuetz EG, Watkins PB, Young B and Guzelian PS, Identification of the cytochrome P-450 induced by macrolide antibiotics in rat liver as the glucocorticoid responsive cytochrome P-450p. *Biochemistry* **24**: 2171–2178, 1985.
10. Kamataki T, Maeda K, Yamazoe Y, Nagai T and Kato R, Sex difference of cytochrome P-450 in the rat: Purification, characterization, and quantitation of constitutive forms of cytochrome P-450 from liver microsomes of male and female rats. *Arch Biochem Biophys* **225**: 758–770, 1983.
11. Imai Y and Sato R, A gel-electrophoretically homogeneous preparation of cytochrome P-450 from liver microsomes of phenobarbital-pretreated rabbits. *Biochem Biophys Res Commun* **60**: 8–14, 1974.
12. Laemmli UK, Cleavage of structure proteins during the assembly of the head of bacteriophage T_4. *Nature (Lond)* **227**: 680–685, 1970.
13. Guengerich FP, Wang P and Davidson NK, Estimation of isozymes of microsomal cytochrome P-450 in rats, rabbits, and humans using immunochemical staining coupled with sodium dodecyl sulfate-polyacrylamide gel electrophoresis. *Biochemistry* **21**: 1698–1706, 1982.
14. Funae Y and Imaoka S, Simultaneous purification of multiple forms of rat liver microsomal cytochrome P-450 by high-performance liquid chromatography. *Biochim Biophys Acta* **842**: 119–132, 1985.
15. Imai Y, Ito A and Sato R, Evidence for biochemically different types of vesicles in hepatic microsomal fraction. *J Biochem* **60**: 417–428, 1966.
16. Nash T, The colorimetric estimation of formaldehyde by means of the Hantzsch reaction. *Biochem J* **55**: 416–421, 1953.
17. Aitio A, A simple and sensitive assay of 7-ethoxycoumarin deethylation. *Anal Biochem* **85**: 488 491, 1978.
18. Meier UT, Kronbach T and Meyer UA, Assay of mephenytoin metabolism in human liver microsomes by high-performance liquid chromatography. *Anal Biochem* **151**: 286–291, 1985.
19. Hayashi S, Noshiro M and Okuda K, Isolation of a cytochrome P-450 that catalyzes the 25-hydroxylation of vitamin D_3 from rat liver microsomes. *J Biochem* **99**: 1753–1763, 1986.
20. Miura T, Komori M, Iwasaki M, Kurozumi K, Ohta K, Ohmori S, Kitada M and Kamataki T, Sex-related difference in oxidative metabolism of testosterone and erythromycin by hamster liver microsomes. *FEBS Lett* **231**: 183–186, 1988.
21. Yasukochi Y and Masters BSS, Some properties of a detergent-solubilized NADPH-cytochrome c (cytochrome P-450) reductase purified by biospecific affinity chromatography. *J Biol Chem* **251**: 5337–5344, 1976.
22. Omura T and Sato R, The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. *J Biol Chem* **239**: 2370–2378, 1964.
23. Phillips AH and Langdon RG, Hepatic triphosphopyridine nucleotide-cytochrome c reductase: Isolation, characterization, and kinetic studies. *J Biol Chem* **237**: 2652–2660, 1962.
24. Lowry OH, Rosebrough HJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
25. Ohmori S, Motohashi K, Kitada M, Kanakubo Y, Igarashi T, Ueno K and Kitagawa H, Purification and properties of cytochrome P-450 from untreated monkey liver microsomes. *Biochem Biophys Res Commun* **125**: 1089–1095, 1984.